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A Rapid and Sensitive Method for the Determination of Phenazone (Antipyrine®) using Gas-Liquid-Chromatography with Nitrogen Detection[1])

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Summary: A simple and rapid method for the quantitative determination of antipyrine, using gas-liquid-chromatography with nitrogen detection, is described. Only one extraction step is needed, the recovery is 91.2% and the precision varies between 3.14–1.96%. The lower limit of quantitative assay reached 0.1 mg/l plasma. During routine handling the method was easy, quick and cheap.

Eine einfache und empfindliche Methode für die Bestimmung von Phenazon (Antipyrin®) mittels Gas-Chromatographie mit Stickstoffdetektor

Zusammenfassung: Eine einfache und schnelle Bestimmungsmethode für Antipyrin im Plasma mittels der Gas-Chromatographie mit Stickstoffdetektor wird beschrieben. Eine einmalige Extraktion ist ausreichend, die Wiederfindungsrate beträgt 91,2%, die Genauigkeit variert zwischen 3,14 und 1,96%. Die Nachweisgrenze liegt bei 0,1 mg/l Plasma. Im Rahmen von Routinebestimmungen hat sich die Methode als spezifisch, zeitsparend und billig erwiesen.

Introduction

Since its synthesis in 1884 antipyrine has been used as an antipyretic and later as an analgesic. From the 1930's it went out of favour as newer analgesics became available. Subsequently antipyrine has proved to be of considerable value as a research tool in pharmacokinetic studies (1).

Brodie & Axelrod (2) described the metabolism of antipyrine and provided much of the evidence on which the antipyrine half-life test as we know it, is based.

Antipyrine® (phenazone; 1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one) is a weak base of $pK_a = 1.4$ which after administration is rapidly absorbed and distributed throughout total body water, with an absorption and distribution normally complete within two hours.

Several methods for the determination of antipyrine in plasma have been published (3–12). The disadvantage of most of these methods is that they are time consuming, expensive and of low sensitivity. The method to be described needs small sample volumes, one extraction step and, using a nitrogen detector, it is sensitive and specific.

Materials and Methods

Apparatus

The gas chromatograms were obtained with a Perkin Elmer 3920 Model Gas Chromatograph equipped with a Thermionic NPD Detector and a Model 56 Potentiometric Recorder (Perkin Elmer Co., Norwalk, Connecticut 06856); the glass column (1.2 m length and 2 mm inside diameter) was filled with 3% Carbowax 20 M on Chromosorb WAW 100/120 mesh (W. Günther Analysentechnik, D-6103 Griesheim).

Peaks were evaluated with an Autolab System I Computin Integrator (Spectra Physics, Santa Clara, Calif. 95051). The extractions were performed with a Model SM shaker (E. Bühler, D-7400 Tübingen).

Reagents

All reagents were of analytical grade. Antipyrine and isopropylantipyrine (for internal standard) were a gift from Hoechst AG (D-6230 Frankfurt).

Procedure

Plasma samples were obtained as described (13). 1 ml plasma was pipetted into a 25 ml glass centrifuge tube, 25 mg/l of the internal standard was added, the solution was alkalinized with 200 µl 5 mol/l sodium hydroxide, and after addition of 6 ml dichlormethane the tube was stoppered and shaken for 15 min;

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the tubes were centrifuged (2500 U/min) for 10 min and the aqueous layer was aspirated and discarded; the organic phase was transferred to a clean centrifuge tube and the solvent was evaporated in a water bath at 40 °C under a nitrogen stream. The residue was three times dissolved in 200 µl dichlormethane and transferred to 1 ml conic vials and the contents were evaporated under a nitrogen stream. The residue was dissolved by vortexing 15 s with 50 µl acetone, and 1 µl of this solution was injected into the gas chromatograph with a Hamilton 701 N microsyringe. The chromatographic conditions were as follows: Helium 30 ml/min; hydrogen 3 ml/min; air 120 ml/min; detector voltage setting 3, and glass bead heating setting at 510; the detector temperature was held at 245 °C, the glass injector liner at 235 °C, and the column at 220 °C; the detector amplifier was used at a setting of range 10 and attenuation 32.

Every day a calibration was made, using plasma at zero time which was spiked with known amounts of antipyrine (5, 10, 25 mg/l) and 25 mg/l internal standard. Concentrations were determined by using the peak area ratio of antipyrine to internal standard, and calculating the regression line (least squares method).

Results

Under the specific conditions in the gas chromatograph (fig. 1) antipyrine had a retention time of 7.10 minutes compared to 4.17 for the standard (isopropylantipyrine). In almost all chromatograms of human plasma a peak preceeding antipyrine was found to have the same retention time as caffeine (5.43 min). This peak did not interfere with the two other ones. A typical standard curve obtained with 5–10–25 mg/l antipyrine is shown in figure 2, with a linear regression line. The precision of the determination of antipyrine is presented in table 1. For three concentrations (5, 10, 25 mg/l) in five determinations the coefficient of variation was 1.96 to 3.14%. The recovery was estimated by adding known amounts of antipyrine and internal standard (20 mg/l) each to 1.0 ml plasma. 91.2 ± 2.1% (n = 5) of the added compounds could be accounted for.

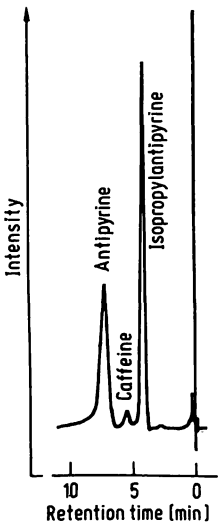

Fig. 1. Gas-liquid-chromatogram from a volunteer plasma by the reported method. The peaks are identified by the retention times:
t = 4.17 min = isopropylantipyrine (internal standard)
t = 5.43 min = caffeine
t = 7.10 min = antipyrine

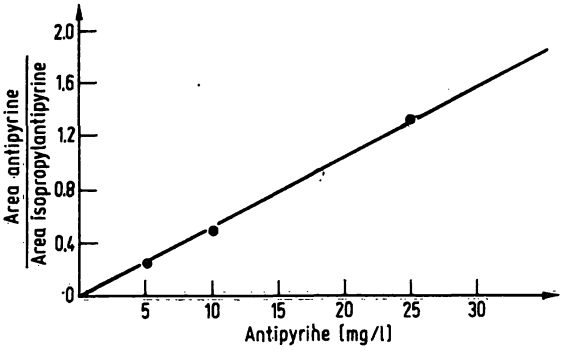

Fig. 2. Typical standard curve obtained with 25 mg/l of internal standard.

Tab. 1. Gas liquid-chromatographic estimation of antipyrine in human plasma. 15 consecutive analyses with the calculation of the mean, standard deviation and the coefficient of variation (CV).

Antipyrine added (mg/l)	found ($\bar{x}$ ± s) (mg/l)	CV (%)
5	5.09 ± 0.16	3.14
10	9.88 ± 0.26	2.63
25	25.06 ± 0.49	1.96

Tab. 2. Plasma concentrations of antipyrine in 10 healthy volunteers 1–13 hours after an oral dose of 15 mg/kg body weight.

Time (h)	Antipyrine $\bar{x}$ (mg/l)	s (mg/l)	range (mg/l)
1	22.8	± 5.2	12.9–32.7
2	20.5	± 4.1	14.7–26.2
5	18.4	± 3.2	13.5–23.4
7	15.5	± 3.1	11.0–19.4
9	14.1	± 3.3	9.8–18.6
11	12.2	± 3.0	8.2–16.4
13	10.7	± 3.1	7.0–14.4

After the oral application of 15 mg/kg antipyrine to 10 healthy volunteers, antipyrine plasma levels were measured between one and thirteen hours. The maximum concentration was 32.7 mg/l, the minimum was 7.0 mg/l (tab. 2).

Blank plasma samples showed no peaks in the region of antipyrine and internal standard.

Discussion

The extraction of antipyrine from plasma described by *Lindgren* et al. 1974 (9), *Huffmann* et al. 1974 (8) and *Eichelbaum & Spannbrucker* 1977 (7) gave, in our hands, inconsistent results, partly caused by the formation of emulsions. The use of dichloromethane as

solvent avoided emulsions and gave an extraction yield of 91.2%. The use of a nitrogen-sensitive detector practically eliminated the solvent peak, gave a straight base line and permitted a better peak integration, thus making the method more specific and sensitive.

As no peak in the blank plasma interfered with the antipyrine or internal standard, the one extraction step was sufficient. The peak with the same retention time as caffeine did not disturb the measurements. Most plasma concentration levels of antipyrine were in the range between 1 and 25 mg/l, but the presented technique could also be applied to samples that contain 0.1 mg/l, simply by increasing the amplifier sensitivity.

In relation to other methods of antipyrine determination, which showed a recovery between 73.2% (*Brinkmann & Hengstmann* (4)) and 93.5% (*Lindgren* et al. (9)) our recovery of 91.2% was good. Also the coefficient of variation of the tested concentration was within the values of 3.9 to 7.9% found by *Lindgren* et al. (9) with their gas chromatographic method.

For us the practicability of the method was verified by the determination of the antipyrine half-life in ten healthy volunteers (13). In routine use, the method was simple, rapid and cheap.

Acknowledgement

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Note added to the revised manuscript:

Drugs tested for interference

There was no interference in the antipyrine assay in patients taking following medications:

azlocillin
cimetidine
clemastine
clonidine
diethylcarbamazine
digoxin
dipyrone
hydrochlorothiazide
penicillin G
phenylbutazone
quinidine
sulfinpyrazone
spironolactone
theophylline
triamterene

The following substances were extracted and did not interfere:

aminophylline
ampicillin
atropine
caffeine
cefazolin
diazepam
diphenylhydramine
dihydralazine
glyburide
ergotamine
furosemide
gentamicine
heptabarbital
lidocaine
methaqualone
metropolol
mezlocillin
neostigmine bromide
pindolol
propanolol
pentazocine
phenobarbital
promethazine
salicylic acid
theobromine
tobramycine
warfarin

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